

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶ :

G01N 27/28, 27/416

A1

(11) International Publication Number:

WO 95/04271

(43) International Publication Date:

9 February 1995 (09.02.95)

(21) International Application Number: PCT/GB94/01690

(22) International Filing Date: 1 August 1994 (01.08.94)

(30) Priority Data:

9315765.9	30 July 1993 (30.07.93)	GB
9410547.5	26 May 1994 (26.05.94)	GB

(71) Applicant (for all designated States except US): CAPTEUR SENSORS & ANALYSERS LTD. [GB/GB]; 66 Milton Park, Abingdon OX14 4RY (GB).

(72) Inventors; and

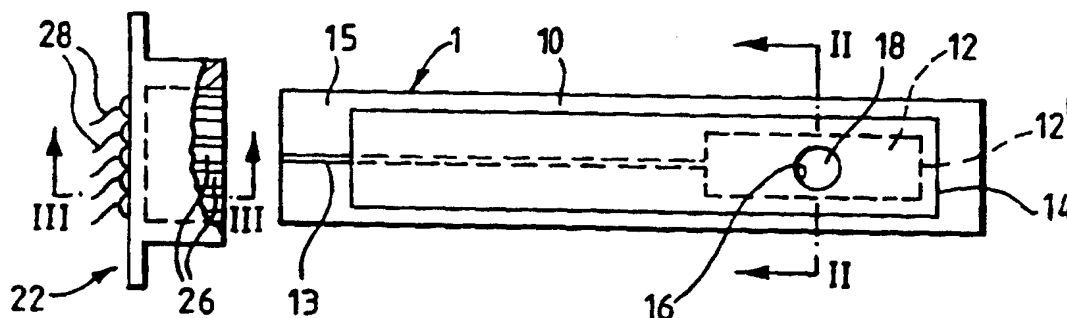
(75) Inventors/Applicants (for US only): WILLIAMS, David, Edward [GB/GB]; 41 Oxford Road, Abingdon OX14 2EE (GB). McGEEHIN, Peter [GB/GB]; School Road, Compton RG16 0QU (GB).

(74) Agent: WENDON, James; Wendon Gregory, Agincourt House, 1 Agincourt Square, Monmouth NP5 3BT (GB).

(81) Designated States: GB, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published*With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: ANALYSIS OF LIQUID SOLUTIONS

**(57) Abstract**

Low concentrations of target species in liquid solutions are analysed using a simple, disposable, electrode sensor element (1) in the form of a flat strip (10) of insulating substrate material with one or more flat electrodes (12) printed on it. The electrode is overlaid with an insulating layer (14) which has a through aperture (16) exposing an active surface (18) of the working electrode (12). The area of the active surface is precisely known. In use, the electrode is vibrated in a plane normal to the plane of the substrate. In preferred arrangements this vibration is intermittent. The sensor, vibrated at a known frequency, produces a stable and repeatable current signal representing concentration of the solution. Any reagents necessary are incorporated in the disposable sensor element as supplied, in a further coating (20) which dissolves when the sensor element is immersed in the solution. Such a coating can also be used to prevent contamination of the active surface (18) before use. The sensor elements may be made in a form in which the electrode working surfaces are exposed only when the element is broken off a larger body, which may be a continuous tape or strip.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ANALYSIS OF LIQUID SOLUTIONS

This invention relates to the analysis of liquid solutions comprising a target species in a liquid solvent, in which the concentration of the target species is measured using a sensor element having at least one electrode which, when in contact with the solution, is caused to generate an electrical current signal, the latter being processed so as to give a measure of the concentration.

The invention relates, in particular, to methods and apparatus for carrying out such analysis, and to sensor elements for use in such methods. It is especially applicable to measurement of low concentrations.

In electro-chemical methods of analysis, the species to be detected is oxidised or reduced at an electrode, and the resulting electrical current is measured. The resulting current depends upon the stirring of the solution. In order to achieve a well-defined and reproducible relation between the concentration of the species to be measured, and the observed current, it is considered in the present art that the stirring must be well-defined, in order to be reproducible. Two methods by which this is done are by the use of a rotating disc electrode, and by the use of a flow channel geometry, in which a uniform laminar flow is maintained past the electrode surface. In general, it is considered that turbulent flow is to be avoided, because it leads to fluctuating and non-reproducible currents.

An alternative to the use of a regulated hydrodynamic regime is to use a microelectrode, in which the geometric size of the electrode is diminished until it

is of the same order as the hydrodynamic or concentration boundary layer thickness at the surface of the electrode. If this is done, then the current becomes independent of the flow regime. A disadvantage is that the measured current, being proportional both to the area of the electrode and the concentration of the target species in solution, might become very small for low concentrations.

In the document WO92/21961, the present Applicants disclosed the use of an electrode in the form of a thin line. That document also disclosed the use of a vibrated line electrode, the effect of the vibration being to cause both an enhancement and a modulation of the measured current, which allowed the rejection of background currents. By these means, measurement of chlorine in water down to concentrations below 1ppm was demonstrated. At these low concentrations, the observed current was smaller than 1nA, and the measurement of lower concentrations was rendered difficult, partly because of the need to measure such low currents. To measure lower concentrations, a larger area electrode might be envisaged: this however requires careful control of the hydro-dynamics, and, indeed this is what the current art teaches.

Instruments currently available, using the electro-chemical method to measure low concentrations of chlorine, feature large (cm^2 scale) electrodes and careful hydrodynamic control. Such devices are incorporated into instruments which are designed only for use in fixed locations. Such instruments also include means for dosing the sample stream with reagents to condition it to the optimum state for the analysis, for example to buffer the pH of the sample

stream, and possibly segregate metallic contaminants, such as Ca^{2+} , Mg^{2+} , Fe^{3+} , and if necessary to precipitate humic acids which might be present. Since chlorine might be present both as free chlorine (which is measured by the electro-chemical instrument), and as forms bound to ammonia (chloramines) (which are not), the system might incorporate the occasional addition of potassium iodide solution, which reacts with all the forms of chlorine, both free and bound, to yield iodine, which is detected by the electro-chemical instrument. The resultant signal therefore gives the total concentration of chlorine, which, by comparison with the concentration of free chlorine, yields the concentration of bound chlorine.

In order to measure very low concentrations, and to distinguish the effects in the current due to the presence of the analytical target species from the effects in the current of processes such as oxidation of the electrode material, the technique of hydrodynamic modulation has been proposed (S. A. Schuette and R. L. McCreary, Anal. Chem. 58 (1986) 1978-82 and 59 (1987) 2692-99). In this method, a well-defined hydrodynamic stimulus was applied, by sinusoidal oscillation of a wire electrode in a direction parallel to the axis of the wire, taking care that the oscillation frequency was such that the flow regime of the electrolyte adjacent to the electrode remained laminar. The resulting sinusoidal oscillation of the current was then detected and used as the analytical signal. These authors also envisaged the use of a double modulation method, combining hydrodynamic modulation at one frequency with modulation of the electrode potential at a different

frequency, in order further to lower the detection limit of the method.

5 In the present state of the art, any system for electro-analysis of liquid solutions must satisfy three particular technical requirements, namely (a) an electrode working surface area which is well defined; (b) control of the hydro-dynamics involved; and (c) an electrode working surface which is clean and active immediately prior to use. Particularly in industrial
10 situations, the need for cleaning and/or preparing the sensor element is at best a nuisance, and may even be impractical and/or too costly to consider.

Accordingly it is attractive to consider the use of disposable sensor elements (electrodes), used once only
15 and then discarded. This in turn leads to two further requirements, namely (d) the electrodes must be as cheap as possible, and (e) they must be repeatable in volume mass production.

Publication W092/21961, mentioned above, discloses an inexpensive, disposable electrode for use in electro-
20 analysis of liquids, in which the working surface is exposed by breaking off a portion of the electrode. Such an electrode can optionally be made such that, if successive portions are broken off when required, the
25 electrode may be used several times.

One object of the present invention is to provide a method and apparatus easily and reliably applicable to measurement of very low concentrations of the target species.

30 Another object is to enable such measurement to be made in situations where separate addition of one or more

reagents would hitherto have been required, but without the need for such separate addition.

5 A further object is to provide a portable electro-analysis apparatus for use in the field, which is simple for use by personnel without a high degree of training.

10 Another object is to provide a sensor element which is cheap, disposable but reliable, which may be an alternative to the type disclosed in the document WO92/21961, or which may be of that type.

Yet a further object is to ensure that the electrodes are protected from contamination before use.

15 According to the invention in a first aspect, a sensor element for use in the analysis of a liquid solution comprising a target species in a liquid solvent, is characterised by:

- a generally planar electrically insulating substrate; and
- 20 - at least one generally planar electrode, carried on the substrate and including or constituting a working electrode which defines an active surface of predetermined surface area, the sensor element having means whereby it can be mounted for application to it of vibration in a direction generally normal to the
- 25 plane of the active electrode surface.

The sensor element preferably has a layer of electrically insulating material overlaid on the substrate and on at least the working electrode, but defining a through aperture for exposing the said

active surface.

The sensor element preferably includes at least one outer layer soluble in a said solvent.

5 Preferably, the, or at least one, said outer layer covers at least the said active surface, so that when it dissolves, the or each electrode is exposed to the liquid.

10 Preferably again, the, or at least one, said outer layer contains at least one reagent for promoting and/or assisting generation of the current signal on the sensor element.

15 According to the invention in a second aspect, apparatus for use in the analysis of a liquid solution comprising a target species in a liquid solvent, is characterised by:

- a sensor element according to the invention in its first aspect;
- a carrier for carrying the sensor element and for making electrical connection thereto;
- 20 - vibrator means for vibrating the sensor element in the said direction; and
- signal processing means for receiving said current signals so as to determine the concentration of the target species in the solution.

25 The apparatus preferably further includes a container for the solution, at least the carrier being mounted on the container such that the sensor element is immersed in the solution in use.

The container preferably includes liquid level control means for determining the amount of solution in the container, and is also preferably portable for use in the field.

5 According to the invention in a third aspect, a method of analysing a liquid solution comprising a target species in a liquid solvent is characterised by the steps, using an apparatus according to the invention in
10 its second aspect, of: vibrating the sensor element in said direction at a known frequency, whereby to produce a stable, repeatable current signal from the sensor element; and processing the current signal so as to determine the said concentration.

15 Preferably, the vibration is applied intermittently, with each period of vibration, of a known duration, being followed by a period of known duration in which no vibration takes place.

20 Preferably also, the area of the said active surface is selected so as to give the sensor element a predetermined sensitivity, being in substantially inverse proportion to a required minimum concentration of the target species to be measured.

25 In some embodiments of the invention, two or more sensor elements may be made as a single unit in the manner disclosed in publication W092/21961. Thus according to an optional feature of the invention, the sensing element has at least one electrode adapted to have a working surface for exposure to an analyte and defining a micro-dimension no greater than about 200
30 micrometres, the electrically insulating substrate with the or at least one said electrode overlaid thereon as

a strip having the said micro-dimension as its thickness, and an electrically insulating layer overlaid on the strip at least in the vicinity of the working surface, the working surface being unexposed and the element further including means for causing a cross-section of the element or elements to become exposed to define the working surface.

With this arrangement, electrode patterns in various configurations can be provided in the form of metallic stripes or strips carried by an insulating substrate, and the aforesaid micro-dimension of the (or each) sensing electrode is achieved by exposing to the analyte a transverse section only of the metallic strip, that is by presenting the edge of the substrate assembly to the analyte liquid, the plain surfaces of the metallic films being protected from contact with the liquid by coatings of insulating material.

The said means comprises at least one line of weakening formed across the substrate and bridged by the electrode or electrodes, whereby, when the substrate is ruptured along a said line of weakening, the or each electrode is also ruptured so as to create its exposed working surface.

The line of weakening is preferably in the form of a groove, which may be formed as part of the substrate manufacturing process, or produced by grinding, scratching or similar methods, or by laser scribing, substrate, preferably a face not carrying electrodes. The groove is positioned so as to facilitate splitting of the substrate along the groove by the user immediately prior to use, so presenting a freshly prepared metal edge surface free from contamination.

A sensor element according to the present invention (in its first aspect), with this additional feature that the working surface of the, or each, electrode is exposed by breaking the substrate immediately prior to use, can be given the soluble outer layer, or at least one soluble outer layer, in the manner explained above, this outer layer containing a reagent which is released into the solution.

The sensor elements can therefore be manufactured in strips or tapes consisting of two or more sensor elements, which are broken off when required for use.

The invention will be more clearly understood on a reading of the description of various embodiments of the invention which follows, and which is given by way of example only and with reference to the accompanying drawings, in which:-

Figure 1 shows a simple form of sensor element according to the invention, together with an edge connector (shown partly broken away above the plane I-I in Figure 3), into which the sensor element is inserted in use;

Figure 2, on a larger scale, is a cross section on the line II-II in Figure 1;

Figure 3 is a cross section through the edge connector, taken on the line III-III in Figure 1;

Figure 4 is a much simplified diagrammatic illustration of one apparatus for use in a method according to the invention;

Figure 5 shows another sensor element according to the

invention, having three electrodes;

Figure 6 is a diagrammatic illustration of a portable electro-analysis apparatus according to the invention;

5 Figure 7 is a diagram showing the variation of signal current from a static electrode subjected to a cycle of electrode potential in a typical weak liquid solution;

Figure 8 shows the effect on the signal current of vibration of the same electrode at four different frequencies;

10 Figures 9 and 10 again show the effect of vibration at four different frequencies, but for respective electrodes having different active surface areas;

15 Figure 11 shows variation of plateau current with vibration frequency for electrodes of four different active surface areas;

Figure 12 shows a typical effect of applying intermittent vibration to a sensor element in one method in accordance with the invention;

20 Figure 13 is a graph showing the variation of the difference in average current as between the vibrated state and the unvibrated state of the sensor element, plotted against vibration frequency;

25 Figure 14 is a graph of the same difference in mean current plotted against the concentration of a solution;

Figure 15 is a graph similar to Figure 14 but for a different electrode and a different solution;

Figure 16 shows a trace of signal current taken over a short period of vibration of a sensor element according to the invention treated with a polymer gel;

5 Figure 17 is a simplified side view showing the junction of two sensor elements made on a common substrate, to be separated from each other before use;

Figure 18 shows a pair of such sensor elements before being separated;

10 Figure 19, on a larger scale, is an end view of one of the sensor elements of Figure 18 after they have been separated; and

Figure 20 shows part of a continuous tape consisting of a series of sensor elements.

15 Reference is first made to Figures 1 to 3 showing a sensor element 1 of a vibratable planar type in accordance with the invention.

20 The element 1 comprises a substrate 10 in the form of a flat strip of a suitable inert and electrically non-conductive substrate material. This may be a synthetic plastics material such as polyvinyl chloride, or a ceramic such as alumina. An electrically conductive layer 12, typically in the form of a conductive ink, is printed in a suitable pattern on one side of the substrate 10. The material of the layer 12,
25 constituting in this example the single electrode of the sensor element, is chosen for its suitability to the application required, and may typically be platinum, gold, silver or carbon. The electrode layer 12 is extended along the substrate 10 by a conductive
30 connecting strip 13 for connecting it with the

measuring circuitry. The area of the printed layer 12 is larger than its active surface 18, which is exposed through an aperture 16 formed in an electrically insulating layer 14 which is applied over the whole of the layer 12 and connecting strip 13, except on the active surface 18 and a short contact area 15 at the other end of the connected strip 13.

The active surface 18 is typically of 10 to 50 mm², and may be in the form of a disc (as shown) or a rectangle. It should be noted at this point that such an electrode shape and area is clearly outside the domain of microelectrodes, and inside the domain for which currently accepted thinking teaches that careful control of hydro-dynamics is necessary if reliable analyses are to be obtained.

The conductive layer 12 is typically applied as a conductive ink, which may for example comprise an epoxy resin filled with the conductive element chosen. The strip 13 may be of the same material as the electrode 12, or may be of another suitable conductive material: typically it is of gold. The insulating layer 14, masking the electrode 12 and strip 13, is preferably applied by printing, and may be of an air cured polymer composition such as an epoxy or silicone resin. In this connection, it should be noted that Figure 2 is somewhat diagrammatic, its vertical scale being greatly distorted so that the various layers can be shown clearly.

In this connection, the active surface 18 in this example is protected from contamination by a coating 20 of a suitable composition which is soluble in the liquid to be analysed. Where the liquid is water, the

protective coating 20 is of a water-soluble and electro-chemically-inactive polymer, such as polyethylene oxide. The layer 20 is not shown in Figure 1, and in Figure 2 it is shown covering not only the surface 18 but also part of the layer 14. Although the necessary cleanliness is absolutely essential only on the working surface 18, it will be realised that the protective coating may if desired cover all or most of the sensor element, including part of the connecting strip 13.

For use, the sensor element 1 is fitted into a suitable edge connector for holding it mechanically and providing the necessary electrical connection. One such connector, 22, is shown in Figures 1 and 3, being in the form of a body of insulating material having a slot 24 containing one or more springy contact strips 26, each of which is connected to a suitable output signal conductor 28. When the element 1 is fitted in the connector 22, the exposed part of the connecting strip 13 lies entirely within the slot 24, and in contact with a contact strip 28.

In use, a disposable sensor element, for example as that just described, is mounted in a space into which a weak solution to be analysed is or will be introduced. In Figure 4, to which reference is now made, this space is indicated diagrammatically within phantom lines 30, the sensor element being indicated at 32. The element 32 is vibrated by suitable means 34 in a direction, indicated by the arrows V, substantially perpendicular to the plane of its active surface 18. Among the effects of this are that mass transport is enhanced in the liquid within the space 30, thus securing dissolution of the water-soluble polymer layer 20 and

so exposing the active surface 18. Another effect of the vibration is that it controls and modulates the mass transport of the analyte to the surface 18. This in turn modulates the detector current generated at the surface 18 as a result of the presence of the species to be analysed in the solution.

The detector current is passed, via the connecting strip 13 and a suitable connection 36, to signal processing means 38, of any suitable known kind, whereby the concentration of the species concerned is measured and/or recorded.

In more elaborate versions, all of the electrodes necessary for an electro-chemical analysis (i.e. a reference electrode and a counter electrode, to complete the circuit) can also be printed on to the substrate, either on the same side or on the opposite side. Figure 5 shows a sensor element 39 modified with separate counter and reference electrodes. The counter electrode 40 may be of platinum, gold or carbon; the reference electrode 42 may be of silver, coated or mixed with silver chloride. If the measured currents are sufficiently small, then the functions of reference and counter electrodes may be combined: a chlorided silver electrode would provide a stable reference potential, whilst a platinum electrode could function as a so-called pseudo-reference electrode, the reliability of which depends on the sensitivity of the analytical signal to small variations of potential, and on the stability of the pH of the solution. Similarly, a silver electrode uncoated with silver chloride can provide a satisfactory reference signal.

Whatever the design of vibratable planar sensor

element, in the method of use described herein it is, as already mentioned, vibrated in a direction normal to the plane of the active surface. The amplitude of the vibration is typically 1mm. The vibration does not
5 need to follow a regular sinusoidal motion: excitation by an eccentric motor-driven cam has been found satisfactory. Any suitable known vibrating means may be used, however.

We have found that, in an intermediate range of
10 vibration frequencies (typically 10Hz to 40Hz), the resulting current does indeed vary in an irregular fashion, as would be expected from current thinking. However, we have been surprised to find that, for frequencies both higher and lower than this range, the
15 mean current is stable over time. The vibration serves to enhance the current measured, this enhancement increasing with increasing vibration frequency until a limit is reached. The modulation of the current, mentioned above, takes place in time with the
20 vibration, but is also surprisingly small.

In order to illustrate these facts, Figure 7 shows, for a platinum electrode (in all the examples given here, platinum electrodes were used) of area 11.4 mm^2 (0.114 cm^2), the variation of current I with electrode
25 potential when a solution containing 3mM Fe(II) is subjected to a cycle of electrode potential at a static electrode. The current shows a characteristic peak-shaped response: the peak arises because of the competition between two effects, namely the increase in
30 current with increasing electrode potential and the decrease in current caused by electrolytic depletion of the electro-active species at the electrode surface. Figure 8 shows the result of an experiment in which

this electrode is vibrated: Figure 8 (a) at 10Hz, 8 (b) at 25Hz, 8 (c) at 40Hz, and 8 (d) at 50Hz.

5 It might be expected that the wagging motion of the electrode would impose a violent swirling movement of the electrolyte adjacent to it, with consequent fluctuations in the current. Contrary to this expectation, at the lower vibration frequency the current rises to a stable plateau; at intermediate frequencies, the current on the plateau does indeed, as
10 would be expected, vary irregularly; but at the highest vibration frequency, the plateau current is again unexpectedly stable. The plateau current increases with increase of the vibration frequency, up to a limit. Figures 9 and 10 show results for similar electrodes of area 1.24 mm^2 ($1.24 \times 10^{-2} \text{ cm}^2$) and 0.076 mm^2 ($7.6 \times 10^{-4} \text{ cm}^2$) respectively, again at four
15 different vibration frequencies f .

Figure 11 shows variation of plateau current with vibration frequency f for the same three electrodes in Figure 11 (a), (b) and (c) respectively, and in Figure
20 11 (d) for a fourth electrode, similar to the others but with an active surface area of 6.1 mm^2 .

Figures 8 to 11 show that for the smaller electrodes, instability of the current is more marked at the lower
25 vibration frequencies. On the larger electrodes, there appears to be a clear rise of the current to a limiting value, with increase of the vibration frequency. The enhancement of the signal induced by the vibration, and the fact that, under particular conditions of vibration the signal is stable, is clearly useful for the
30 purposes of analytical chemistry.

The proposals in the paper by Schuette et al have been mentioned above, in particular careful control of the hydro-dynamics to enable usable signals to be produced. By contrast, in the method described herein, there is
5 no need for such close control because, although the current oscillations imposed upon the steady state current by the vibrations are complex in form, they are periodic with the motion. The techniques of phase-sensitive detection can indeed be used to extract the
10 modulated part of the current, and thereby to distinguish that part which is controlled by transport of a reagent to the electrode surface (the desired analytical signal) from that part which is not (the background current).

15 The method described in the document W092/21961 mentioned above, in a patent application of the present Applicant, is also found, surprisingly, to work very well in the practice of the present invention. In that method, what is measured is the difference in current
20 between that flowing when the electrode is stationary and the mean value when the electrode is vibrating.

Whilst it has been found possible, on an electrode of area 6.1 mm^2 (0.061 cm^2), to observe directly above the background the additional current due to oxidation of
25 Fe(II) at a concentration of $1 \mu\text{M}$, the currents were small in comparison with the background. This would clearly cause difficulties in an analytical determination, since it could never be guaranteed that the background would remain constant.

30 In this connection, reference is now made to Figure 12, which shows the response of the current to an electrode of area 0.061 cm^2 as a consequence of the presence in

the solution of $1\ \mu\text{M}$ (Fe(II)), when the vibration is alternately switched on (for periods indicated by X) and off. Surprisingly, the current with the vibration switched off did not continuously decay at a rapid rate, as would normally be expected for current controlled by diffusion to a stationary planar electrode. It is assumed that the movement of the electrolyte caused by the vibration persisted sufficiently to counter this decay on the time scale of the measurement. It is again surprising that, given that such movements might be expected to induce an irregularity in the current, no such irregularity has in fact been observed. It should however be noted that, should any such irregularity appear and cause a difficulty, the results given in Figure 11 show that a difference could equally well be taken between two different states of vibration, one at a high frequency and the other at a low frequency.

Figure 13 shows that, at a low concentration of $1\ \mu\text{M}$, in contrast to the behaviour to be deduced from Figure 11 relating to the high concentration of 3mM , the difference ΔI in average current between the vibrated and un-vibrated state varies approximately linearly with the vibration frequency f .

Figure 14 shows linear variation, against the concentration of hexacyanoferrate in water, of the difference ΔI in mean current (produced by an electrode according to the present invention with an active surface area of $6.1\ \text{mm}^2$), between vibrated and un-vibrated states, at low concentration and at a fixed vibration frequency of 50Hz . This value is chosen to be high enough, with the electrode area used, for the current not to fluctuate widely. Figure 14 also

indicates that, at higher concentration, the current difference increases sharply with further increase of concentration. It is evident from Figure 14, and has been confirmed by separate experiment, that the limit of detection by this method, with the instrumentation used and the particular electrode area employed, was around 0.1 μM .

It will be clear from the foregoing that the active surface area of the working electrode of the vibratable planar sensor element of this invention can be increased without loss of the properties of the sensor element. Therefore, the detection limit can be reduced still further. It will be further evident that, at higher concentration of the target species for which the analytical current is much larger than the background, there is no need to use the differencing procedure described above, since the value of the stable current resulting from vibration at a sufficiently high frequency is directly proportional to the concentration of the target species.

In other words, the active surface area of the electrode is chosen according to a predetermined minimum concentration of target species, this area being inversely proportional to the said minimum concentration.

An important analytical task is the measurement of chlorine in water at low concentration, for which there is a need for simple methods that can be practised by little-trained practitioners, away from a laboratory. Reference is accordingly now made to Figure 15, which shows the result of current difference measurements of the type measured above, using electrodes of smaller

area (4 mm^2 or 0.04 cm^2) applied to low-concentration solutions of chlorine in water, to which a phosphate buffer, pH 7.4, had been added. Evidently, concentrations below $1 \mu\text{M}$ (0.071 ppm) can of course be measured by this means: determination of such lower concentrations would simply require an electrode of larger area.

In the electro-chemical measurement of species such as chlorine in water, one difficulty in the development of field analyses is the need to condition the solution. In order to increase its conductivity sufficiently, it is buffered at a fixed pH; and to segregate any metal ions which might interfere with the analysis, a complexing agent, such as ethylenediaminetetra-acetic acid (EDTA), is added. Such reagents can be mixed with a water-soluble polymer (such as the hydroxyethylcellulose powder supplied by Aqualon GmbH, P.O. Box 130125, Paul Thomas Strasse 58, D-4000 Düsseldorf 13, Germany, under the Trade Mark NATROSOL), so as to form a paste. We have now found that if such a paste is overlaid (typically by printing) on or around the active surface of the electrode, and allowed to dry to a stable film, this film will redissolve rapidly and easily into the solution when the electrode is inserted into the solution to be analysed, and that this re-dissolution is substantially aided by the vibration of the electrode. Vibration of the electrode also serves to secure rapid mixing of the reagents into the solution.

Example (free chlorine): Solutions of chlorine in water were prepared, of concentration nominally $30 \mu\text{M}$, using photolysed, triple-distilled water: no salts were added to the solution to impart electrical

conductivity or buffer capacity, and direct electro-chemical determination of the concentration of chlorine in the solution therefore proved impossible. A buffered, printable polymer gel was prepared: to
5 20 cm³ of a concentrated phosphate buffer solution (containing 0.68 g potassium dihydrogen phosphate and 1.14 g potassium hydrogen phosphate) "Natrosol" was added in a quantity sufficient to produce a fluid gel that could be applied to surfaces using a Pasteur
10 pipette. Screen-printed platinum electrodes were warmed on a hotplate, and several applications of the buffered polymer gel were made to the surface of the insulator around the active electrode area, with intermediate drying. The result was a thick film of
15 the dry gel, surrounding the active area.

A resulting electrode was introduced into 20 cm³ of the chlorine solution, along with a silver wire as a quasi-reference electrode and a platinum wire as a counter electrode. The electrodes were introduced to the
20 solution, with the potentiostat switched on and set for a working electrode potential of +0.20 V with respect to the silver wire. Vibration was then started, at A in Figure 16 (to which reference is now made). This was continued for 30 seconds, and stopped where
25 indicated at B in Figure 16.

The resulting current record, shown in Figure 16, shows that no analytically significant current flowed immediately upon the introduction of the electrode; however, after a short interval (presumably sufficient
30 for the dissolution of the reagent-loaded polymer), a strong reduction current appeared. The difference between the current with and without vibration, with reference to the calibration curve given in Figure 15,

indicates a chlorine concentration consistent with the amount of chlorine added to the solution, and thus demonstrates the equivalence of the method in which reagent is added from a water-soluble polymer to that in which reagent is added separately to the solution. It is clear that the presence of the polymer had no deleterious effect on the electro-chemical signal.

It will be realised that such a reagent-loaded polymer layer could be applied by a number of production methods known in the art, for example screen-printing or spin-coating, and further that it could be deposited to cover the active electrode surface. In this case it would provide the additional function of protecting the active surface from adventitious contamination, during handling before use. Accordingly, this is a preferred form of the protective layer 20 in Figures 1 and 2.

It is well known that total chlorine can be measured by the addition of potassium iodide, whereupon the chlorine present (whether free or combined) reacts to form iodine, which may be electro-chemically determined. Therefore, it may be conceived to incorporate, into the water-soluble polymer layer, potassium iodide along with the buffer. The measured current upon immersion of the electrode and vibration would then yield the total chlorine content of the solution.

An application of the method of the invention, in which free and bound chlorine are measured using a single sensor element (or analysis strip) in accordance with the invention, will now be described.

The sensor element is for example as shown in Figure 4,

having the protective coating 20 (Figure 2). Here, however, the coating 20 consists of two soluble polymer layers. One of these layers is printed only on to the electrode surround. The other layer may be printed
5 over the top of the first layer and also optionally over the electrodes. The two polymers are chosen to differ in their rate of dissolution, for example by choice of substituents, or by cross-linking of one of them, for example by radiation. Alternatively, the two
10 layers may comprise different polymers: the more rapidly dissolving layer may for example be "Natrosol", whilst the more slowly dissolving layer may for example be of a substituted polyethylene oxide; such compounds are known as controlled-release agents for delivery of
15 pharmaceuticals. The first, slower-dissolving, layer is dosed with potassium iodide; the second, rapidly-dissolving, layer, optionally on top of the first and on top of the electrode, contains the buffer. Then, upon immersion and vibration, the electro-chemical
20 signal will first show the concentration of free chlorine. Later, as the second layer dissolves, releasing potassium iodide into the solution, the signal would increase to a value indicating the total concentration of chlorine present.

25 An application of the method of the invention to the measurement of ammonia in aqueous solution will now be described with reference to Figure 6. Figure 6 shows, diagrammatically, a container 60 for containing water
30 62 to be analysed. The container has means for maintaining the water surface 64 at a predetermined level: in this example this comprises an overflow 66 acting as a weir. At a suitable fixed location below the level 64, a measuring unit 68 is fitted sealingly

in an orifice 70 of the container. The unit 68 comprises a body 72, carrying a vibrator which is coupled to a connector 74 (for example like the connector 22 in Figures 1 and 3), into which is fitted
5 a disposable sensor element 76 of the vibratable planar kind described above. The depth to which the electrodes 78 of the element 76 are submerged is thus defined. The unit 68 is coupled to suitable signal current measuring means 80.

10 The element 76 is in this example generally as shown in Figure 5; but here, one of the electrodes 40, 42 referred to here as the second electrode is a combined reference and counter electrode. The other electrode (42 or 40), referred to here as the third electrode, is
15 such that chlorine can be generated on it at a known rate and in known amount when a known current is passed for a known time. The sensing element has a water-soluble polymer coating 20, this coating carrying a suitable buffer to control the solution pH, with enough
20 sodium chloride to render the solution sufficiently concentrated for chlorine to be evolved with high current efficiency, and, if necessary, a complexing agent such as EDTA to sequester excess metal ions.

One of the means by which chlorine is combined in
25 solution is as chloramines, formed by reaction with dissolved ammonia. The reaction may be slow, particularly at low concentration of chlorine and ammonia, with the rate dependent upon both concentrations. In this method, therefore, the
30 reaction of chlorine with ammonia is used as a measure of the concentration of ammonia. This method is particularly attractive to use because, in the water industry, one of the main reasons for determination of

ammonia is to be certain that sufficient chlorine is added to obtain disinfection.

5 Using the apparatus of Figure 6, the sensing element 76 is vibrated so as to dissolve the reagents in the coating 20 and mix them with the solution 62. With the vibrator working, a current is passed between the third and second electrodes so that a known amount of chlorine is generated and mixed into the solution. Vibration is carried out intermittently as described above with reference to Figure 12, and the first electrode is polarised with respect to the second electrode.

15 Both the resultant chlorine concentration, and its rate of decrease, can thus be determined. The ammonia concentration is determined both from the total amount of chlorine required to be added in order to produce a detectable concentration of free chlorine (the chlorine demand), and from the rate of consumption of chlorine.

20 The method according to the invention may also be used for measuring heavy metals in water. This embodiment of the method is based on the well-known technique of anodic stripping voltammetry, with initial co-deposition of mercury in order to form, in situ, a mercury drop electrode. The sensor element has a first electrode, preferably of platinum or carbon, and a second electrode which is preferably of chlorided silver, or silver or platinum. The reagent coating (20, Figure 2) contains a soluble mercury salt, such as mercurous nitrate together with a buffer, and (optionally) suitable complexing agents to allow separation of different metals. A container of defined volume is provided, as above. Although it is not

essential that the volume be precisely fixed, it must not be so large that the reagents dissolved from this coating are unable to form a sufficiently concentrated solution to allow the analysis to proceed.

- 5 In use, the sensor element is vibrated so as to dissolve the reagents and mix the solution. Still under vibration, the first electrode is polarised for a fixed time as a cathode with respect to the second electrode, causing the deposition of mercury together with other heavy metals which might be present. These
10 metals become dissolved in the deposited mercury. Finally, the electrode potential is caused to rise in a linear fashion with time, in the anodic direction. As the deposited metals redissolve, a series of peaks is
15 observed in the current in the anodic direction. The amount of metal deposited, determined from the area enclosed by the peak of current observed upon redissolution, is directly proportional to the concentration originally present.
- 20 It will be realised that numerous other applications for versions of the method according to the invention will suggest themselves to persons working in the field of electro-analysis of liquids, especially that of weak liquid solutions; and that the sensor element used can
25 be adapted in many ways, within the scope of the invention, to suit the requirements of each particular application. For example, it may have one, two, three or even more electrodes. Any suitable electrode materials may be used: for instance in the description
30 with reference to Figure 5, and in the example of a method of measuring heavy metals in water, a good electrode material, highly tarnish resistant, is a palladium-silver alloy, typically containing 20%

palladium.

Embodiments of the invention in which two or more sensor elements according to the invention are manufactured in one piece, so that individual elements
5 can be broken off for use as required, will now be described with reference to Figures 17 to 20. Although these are described in connection with the assessment of chlorine in solution, it will be understood that the person skilled in electro-analysis will readily adapt
10 such elements, and their methods of use, to the analysis of other liquid solutions.

Figure 17 shows in a simple form part of an article in the form of a pair of sensor elements as disclosed in publication WO92/21961. A flat insulating substrate
15 110 has at least one thin metallic electrode 116 deposited on it and overlaid with an insulating layer 120. The electrode 116 and insulating layer 120 bridge a weakening groove 112 formed in the substrate. Immediately before use, the article is broken along the
20 groove, i.e. along the transverse plane AA, to expose a cross sectional surface of the element, and thus a cross sectional working surface of the electrode 116. The thickness T of the element is comparable to or less than the concentration boundary layer thickness of the
25 solution. This exposed working surface is the only part of the electrode in contact with the solution.

Figures 18 and 19 show a version of such an article comprising a pair of sensor elements 130, 132, for use in the measurement of free and bound chlorine in
30 aqueous solution. The technique of such measurement has already been discussed above, for the case where a single sensor element has two soluble reagent-

containing layers enabling free and total chlorine to be measured one after the other. This embodiment provides an alternative form of sensor element for this purpose.

5 In Figures 18 and 19, the common substrate 110, with its weakening groove 112 across the middle, in a plane BB has electrodes 136 printed on its upper surface in a working zone 134 straddling the transverse plane BB that contains the groove 112. This working zone
10 consists of any number of suitable layers. By way of example, these comprise: a fired dielectric layer 139 covering the electrodes 136 (which are of gold in this example); a palladium electrode 140 printed on the layer 139 and itself covered by a polymer carbon layer
15 142; a polymer dielectric layer 144 overlying the layer 142; and an outer layer 146 or 147 applied on the layer 144. The electrodes are connected to electrical contacts 138 in the usual way.

The outer layer 146 or 147 is of a soluble compound as
20 described earlier, containing a reagent for release into the solution. Thus the layer 146 typically contains the buffer and the layer 147 potassium iodide. In use, the two sensors are separated by rupture along the plane BB, and are immersed in the chlorine
25 solution. Free and total chlorine are measured simultaneously by the sensors 130 and 132 respectively as the buffer and iodide are simultaneously released.

As can be seen from Figure 18, in this example each
30 electrode has an electrical connection on each side of the line of weakening 112, the latter being disposed along the mid-line of the substrate, i.e. in the plane of symmetry, so then when it is broken, a pair of

substantially identical sensors 130 and 132 are formed.

Numerous modified forms of these electrodes can readily be conceived. For example, two pairs of sensors may be provided, one pair having a single buffer layer 146
5 over the whole of its working zone 134, and the other having a single iodide layer 147 over the whole of its working zone. One of these pairs can be used (after separation) for determining free chlorine only, and the other for total chlorine only, the second element in
10 each pair being used to provide duplicate measurements for validation purposes.

It will be understood that the composition, location and purpose of each electrode in each sensor will be chosen to suit the particular application, the three
15 electrodes in Figures 18 and 19 being purely illustrative.

Figure 20 shows a continuous tape comprising an insulating tape substrate 200, having electrical contacts 202 applied on it alternately with working
20 zones 204, the contacts 202 being joined to the electrodes (not shown) in the zones 204 by connecting strips 206 in the manner described earlier herein. Lines of weakening are provided in the transverse planes CC, through the contacts 202, and DD through the
25 working zones, so that each sensor (sensor element), or pair of sensors (sensor elements), can be broken off the tape when required.

Although Figure 20 shows a tape in which buffer layers 146 and iodide layers 147 are both present, the soluble
30 outer layers of all the working zones in any one tape are preferably the same, e.g. containing either buffer

- or potassium iodide. The analyst will then have a supply of sensors on two tapes, one with a soluble buffer layer on all its sensor elements for measurement of free chlorine as required, and the other with a
- 5 soluble iodide layer on all its sensor elements, for measurement of total chlorine as required.

As before, the difference between the concentration of total chlorine and that of free chlorine is taken to represent the bound chlorine present.

- 10 It will be understood that Figure 20 can equally represent part of a strip (rigid or semi-rigid), instead of a tape, carrying a plurality of sensors to be broken off the strip as required.

CLAIMS

1. A sensor element (1; 39) for use in the analysis of a liquid solution comprising a target species in a liquid solvent, characterised by:
- 5 - a generally planar electrically insulating substrate (10); and
- at least one generally planar electrode (12; 18, 40, 42), carried on the substrate and including or constituting a working electrode (12; 18) which
- 10 defines an active surface (18) of predetermined surface area, the sensor element having means (15) whereby it can be mounted for application to it of vibration in a direction (V) generally normal to the plane of the active electrode surface (18).
- 15 2. A sensor element according to Claim 1, characterised by a layer (14) of electrically insulating material overlaid on the substrate and on at least the working electrode, but defining a through aperture (16) for exposing the said active surface
- 20 (18).
3. A sensor element according to Claim 1 or Claim 2, characterised by at least one outer layer (20) soluble in a said solvent.
4. A sensor element according to Claim 3,
- 25 characterised in that the, or at least one, said outer layer (20) covers at least the said active surface (18), so that when it dissolves, the or each electrode (12; 18, 40, 42) is exposed to the liquid.
5. A sensor element according to Claim 3 or Claim 4,

characterised in that the, or at least one, said outer layer (20) contains at least one reagent for promoting and/or assisting generation of the current signal on the sensor element.

5 6. A sensor element according to any one of the preceding Claims, characterised in that it has at least one electrode (116, 136, 140) adapted to have a working surface for exposure to an analyte and defining a
10 micro-dimension (T) no greater than about 200 micrometres, the electrically insulating substrate (110, 200) having the or at least one said electrode overlaid thereon as a strip having the said micro-dimension as its thickness, and an electrically
15 insulating layer (120, 144) overlaid on the strip at least in the vicinity of the working surface, the working surface being unexposed and the element further including separation means (112) for causing a cross-section of the electrode or electrodes to become exposed so as to define the working surface.

20 7. A sensor element according to Claim 6, characterised in that the separation means comprises at least one line of weakening (112) formed across the substrate (110, 200) and bridged by the electrode or electrodes (116, 136, 140), whereby, when the substrate
25 is ruptured along a said line of weakening, the or each electrode is also ruptured so as to create its exposed working surface.

30 8. A sensor element according to Claim 7, characterised in that the or each electrode (136, 140) has an electrical connection (132, 202) on each side of the line of weakening (112), the latter being so disposed that when it is broken, the sensor element

becomes a pair of substantially identical sensors (130, 132).

5 9. A tape or strip comprising a continuous insulating substrate (200), characterised in that the substrate constitutes the substrate for a plurality of sensor elements according to any one of Claims 6 to 8, so that a sensor element can be broken off the tape or strip as required.

10 10. Apparatus for use in the analysis of a liquid solution comprising a target species in a liquid solvent, characterised by:

- a sensor element (1; 39; 76) according to any one of Claims 1 to 8;
- 15 - a carrier (22; 74) for carrying the sensor element and for making electrical connection thereto;
- vibrator means (34) for vibrating the sensor element in the said direction (V); and
- signal processing means (38; 80) for receiving said current signals so as to determine the concentration of
20 the target species in the solution.

11. Apparatus according to Claim 10, further characterised by a container (30; 60) for the solution, at least the carrier (22; 74) being mounted on the container such that the sensor element is
25 immersed (1; 39) in the solution in use.

12. Apparatus according to Claim 11, characterised in that the container (60) includes liquid level control means (66) for determining the amount of solution in the container.

13. Apparatus according to Claim 11 or Claim 12, characterised in that it is portable, for use in the field.

5 14. A method of analysing a liquid solution comprising a target species in a liquid solvent, characterised by the steps, using an apparatus according to any one of Claims 10 to 13, of vibrating the sensor element (1; 39; 76) in said direction (V) at a known frequency, whereby to produce a stable, repeatable current signal
10 from the sensor element; and processing the current signal so as to determine the said concentration.

15 15. A method according to Claim 14, characterised in that the vibration is applied intermittently, with each period of vibration, of a known duration, being followed by a period of known duration in which no vibration takes place.

20 16. A method according to Claim 14 or Claim 15, characterised in that the area of the said active surface (18) is selected so as to give the sensor element (1; 39; 76) a predetermined sensitivity, being in substantially inverse proportion to a required minimum concentration of the target species to be measured.

25 17. A method according to any one of Claims 14 to 16, characterised by the use of a pair of sensors (130, 132) for simultaneous measurement of free and total chlorine in the solution, each said sensor having an exposed outer layer (146, 147) soluble in the solution, with the said outer layer (146) of one sensor (130)
30 containing a buffer and the outer layer (147) of the other sensor (132) containing an iodide, whereby the

sensors give output signals representing the concentration of free chlorine and total chlorine, respectively, as the said outer layers are dissolved in the solution.

1/7

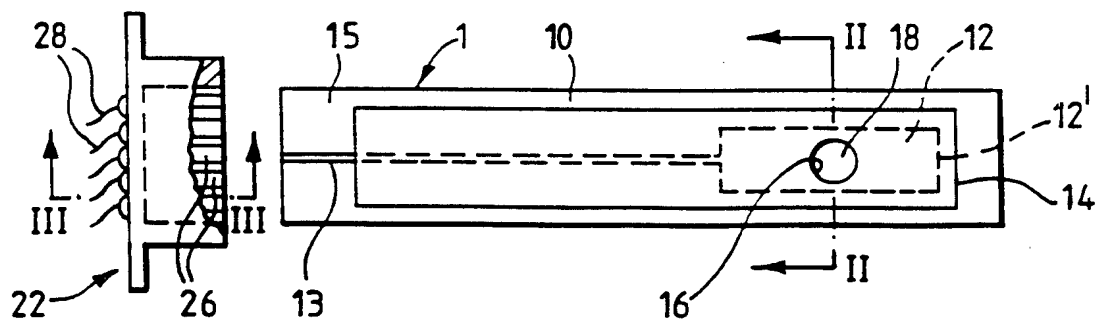


Fig.1.

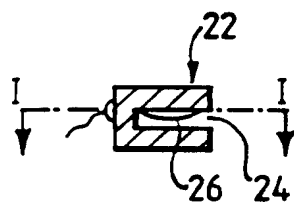


Fig.3.

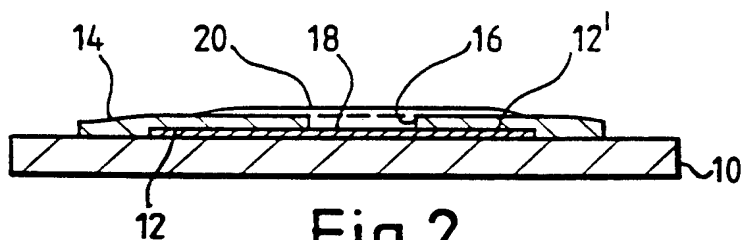


Fig.2.

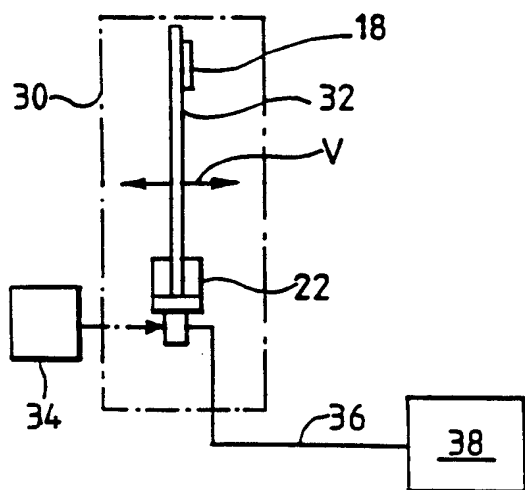


Fig.4.

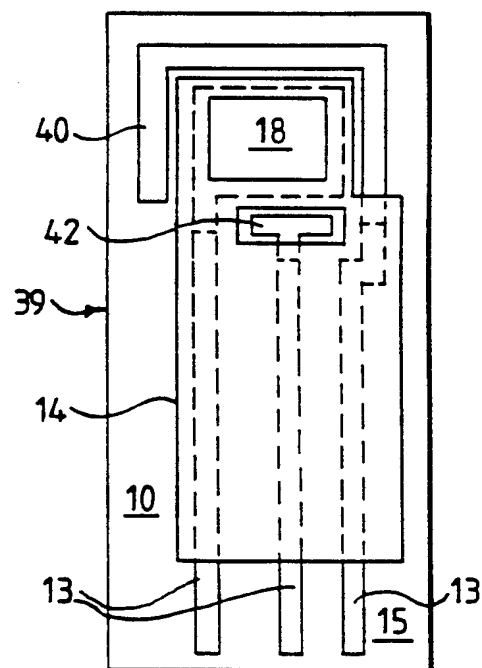


Fig.5.

2 / 7

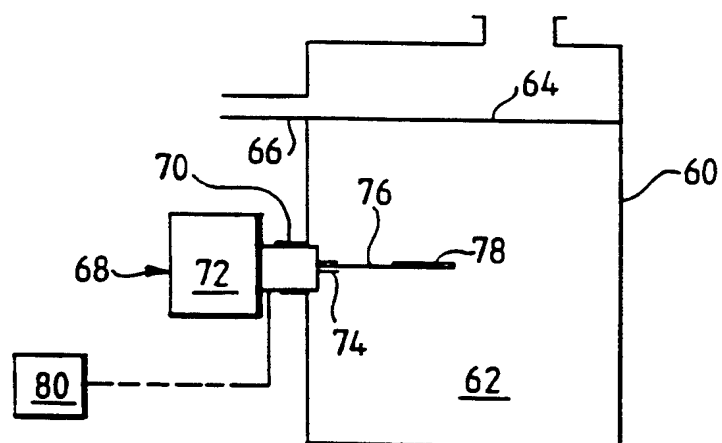


Fig.6.

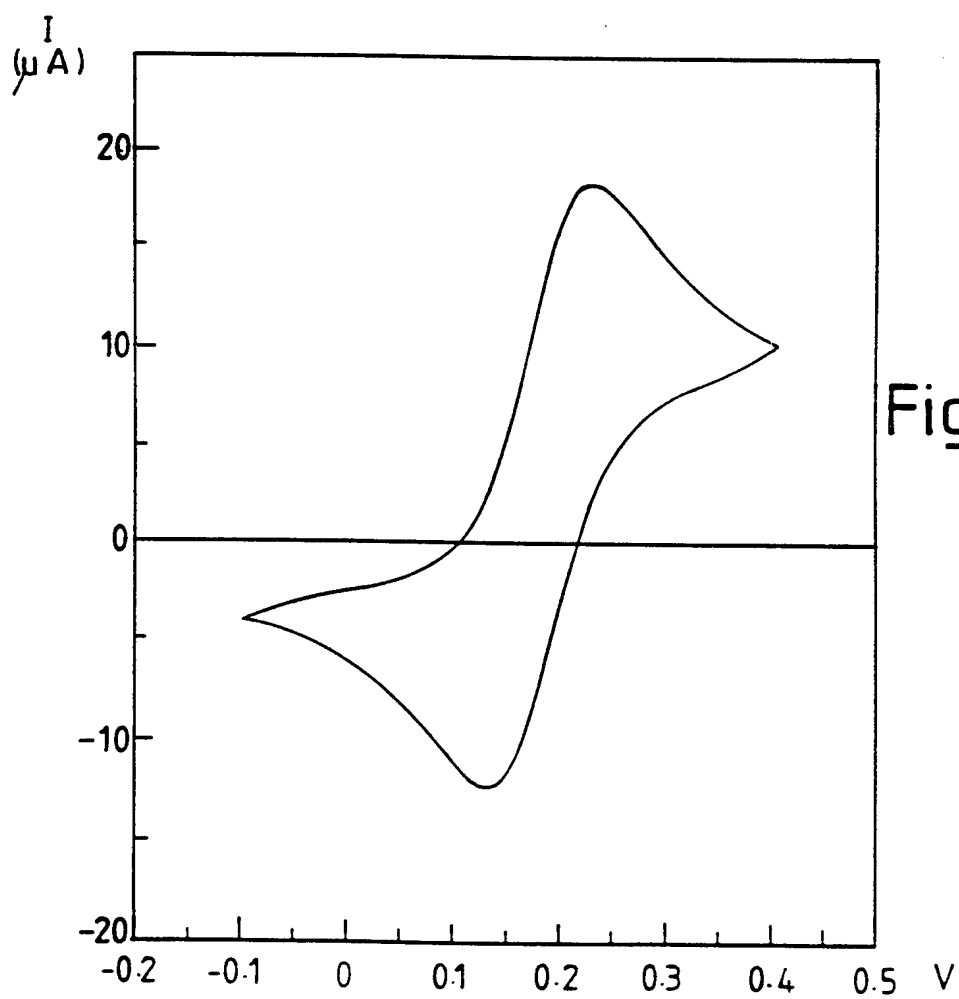


Fig.7.

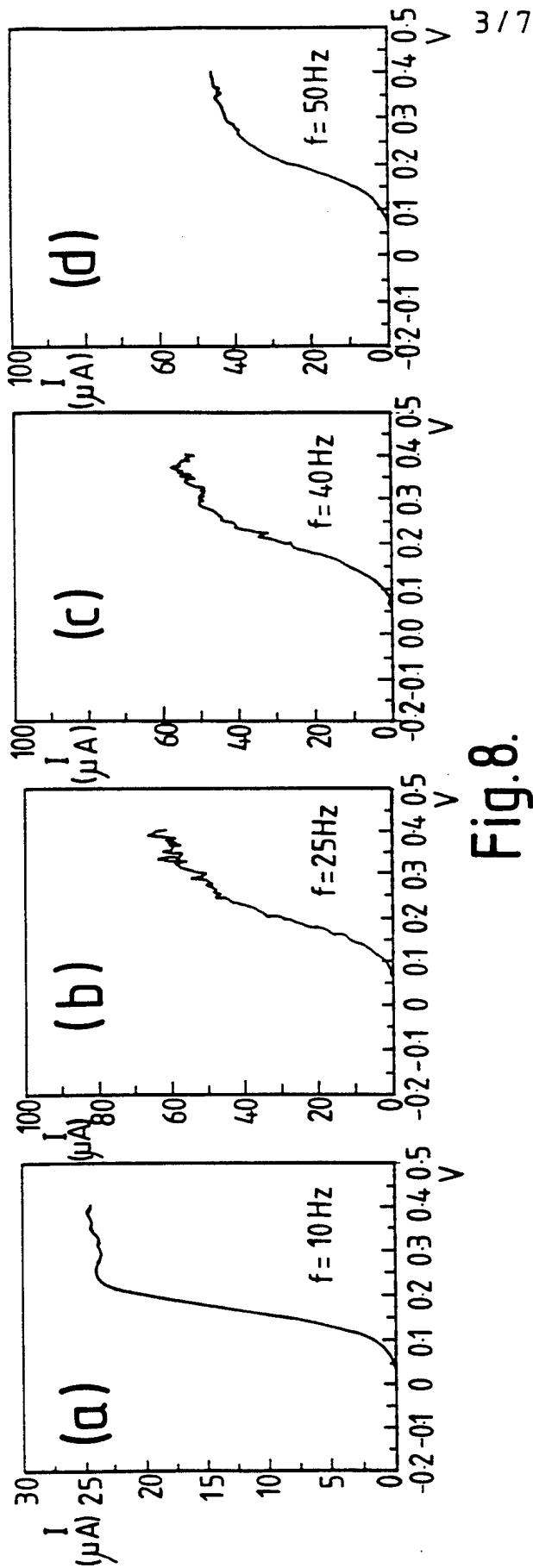


Fig.8.

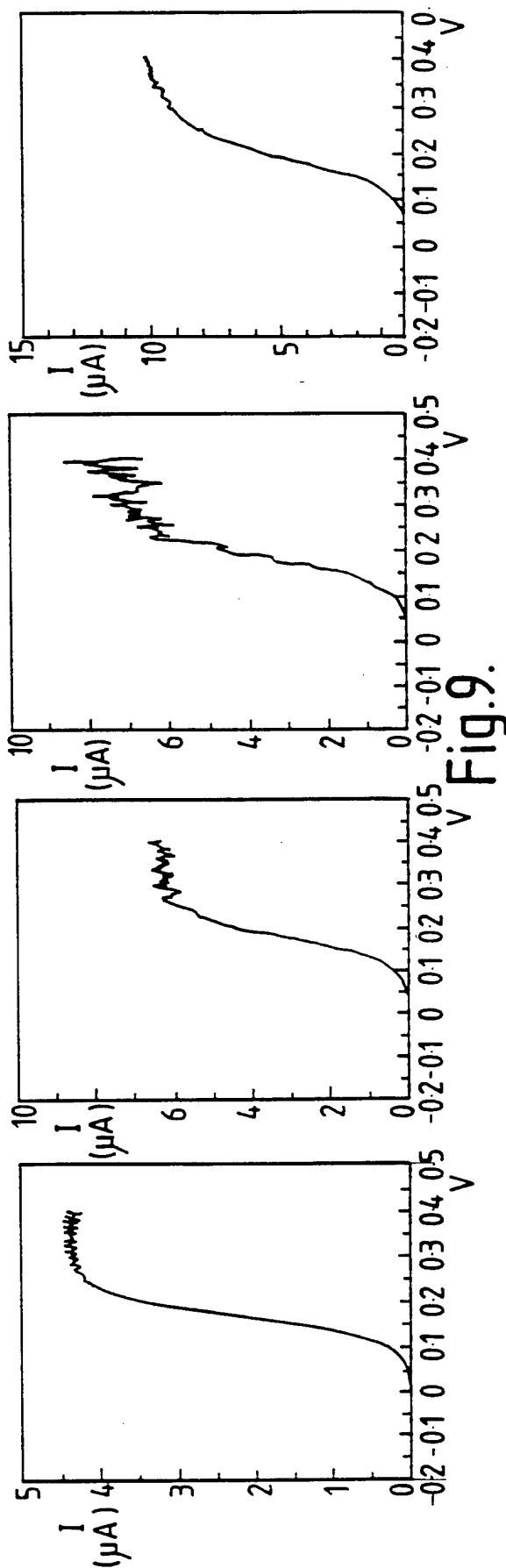
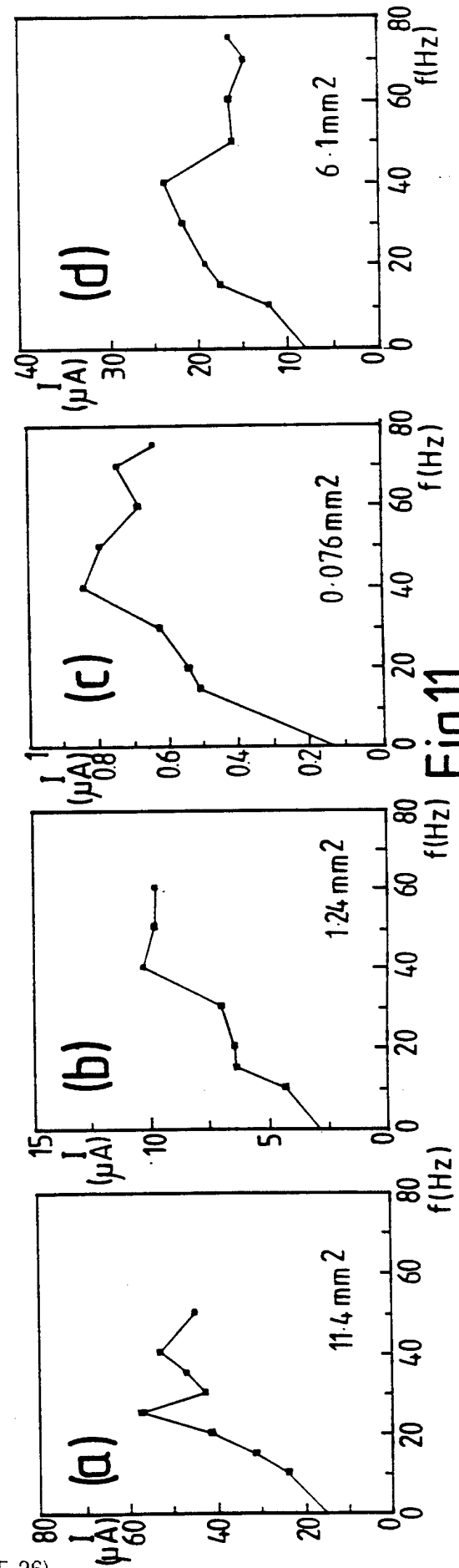
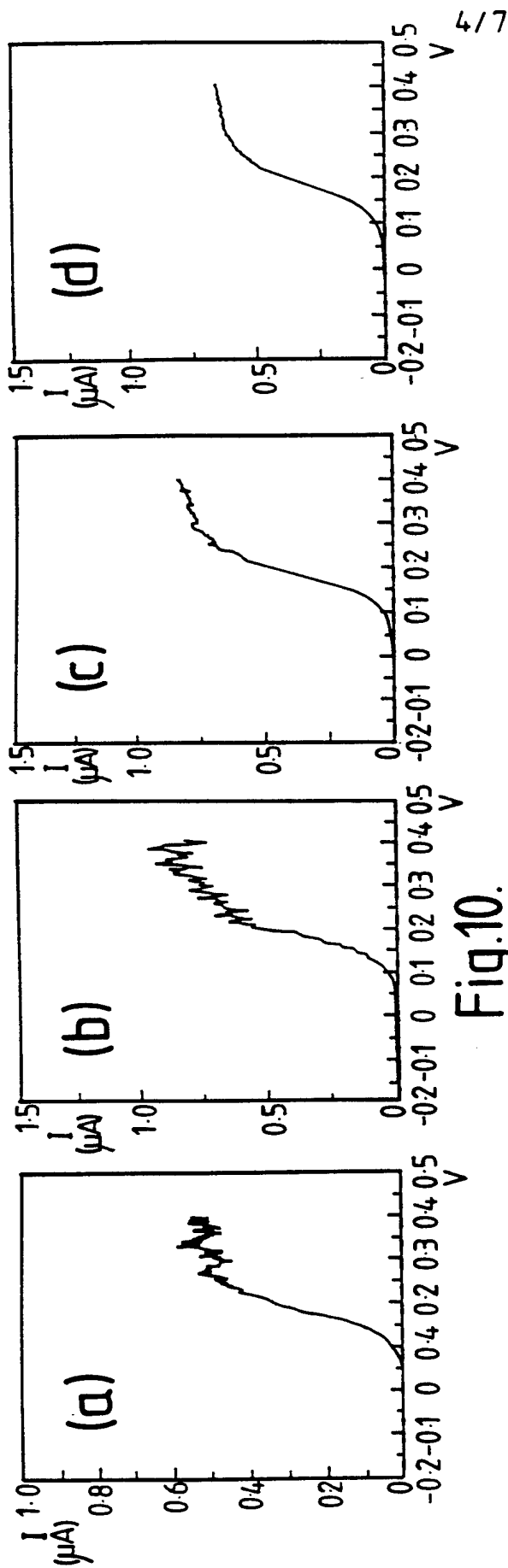


Fig.9.



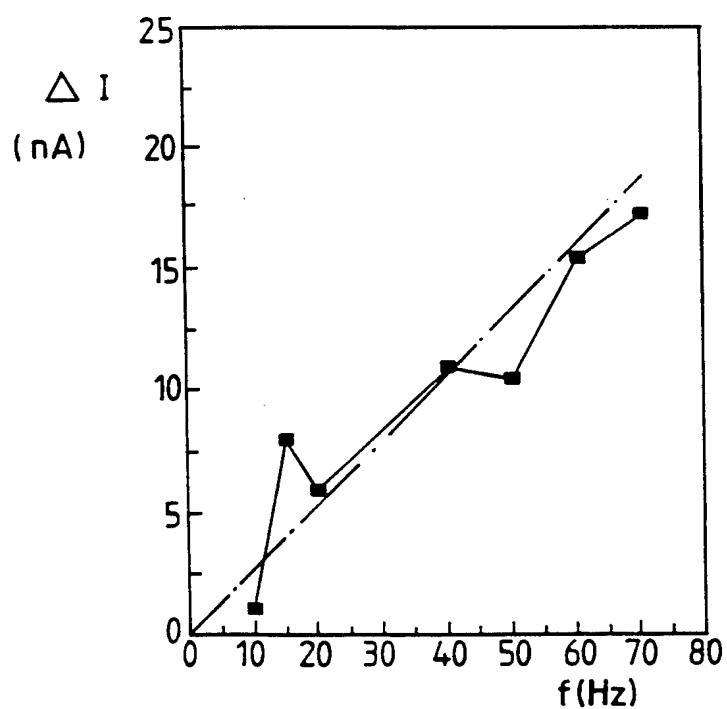
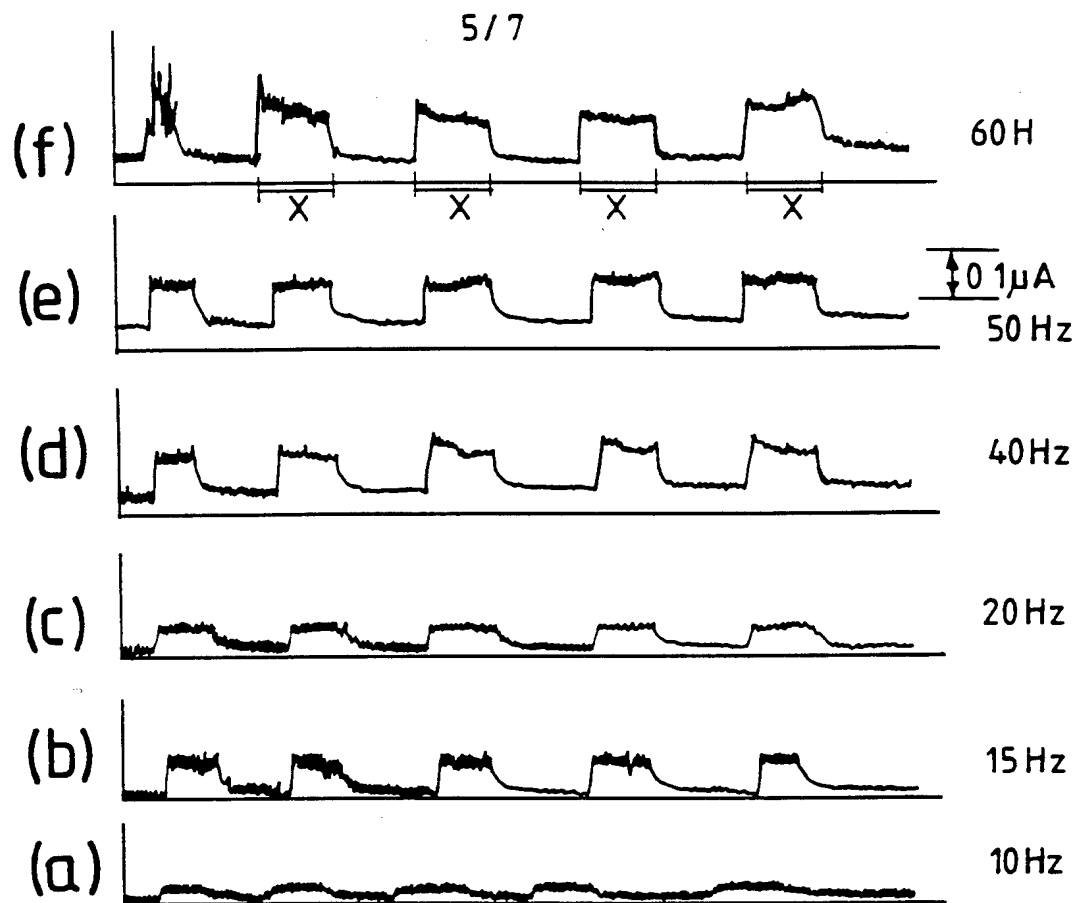


Fig.13.

6 / 7

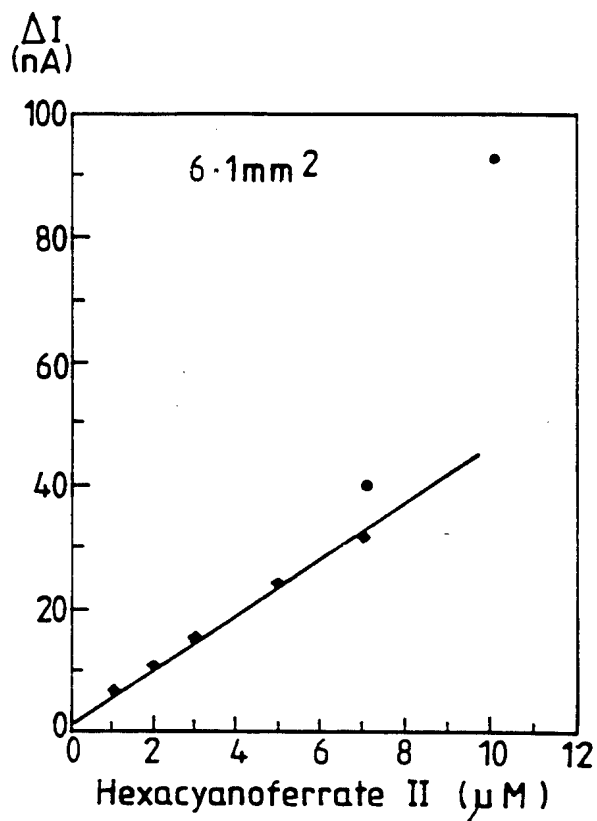


Fig.14.

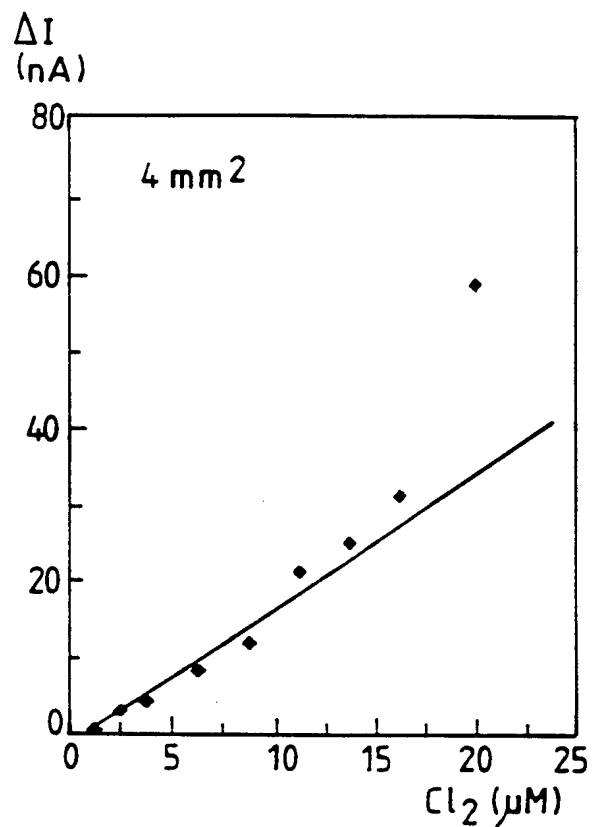


Fig.15.

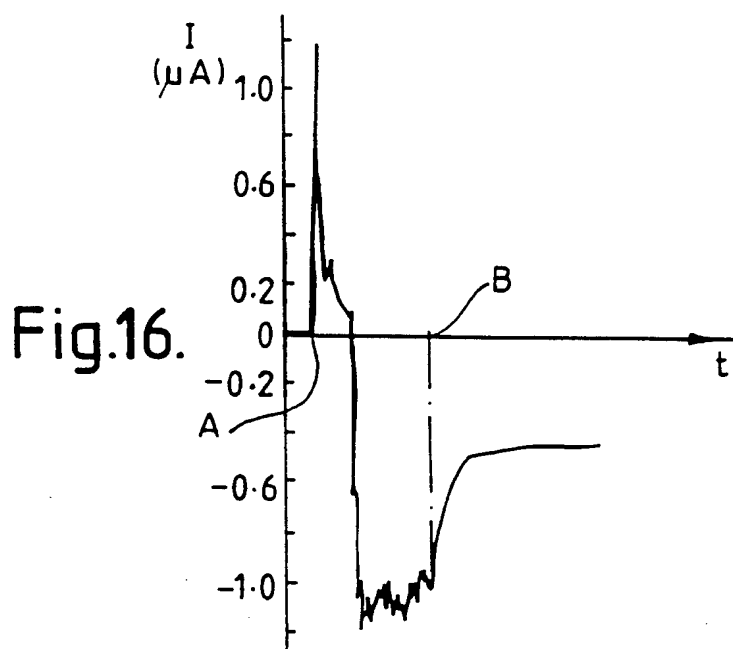


Fig.16.

7/7

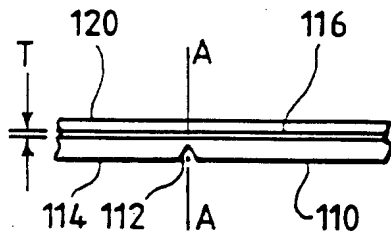


Fig.17.

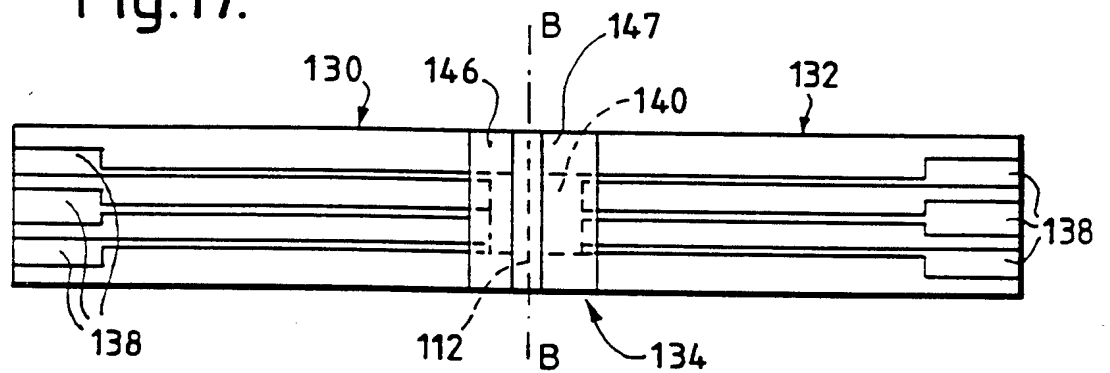


Fig.18.

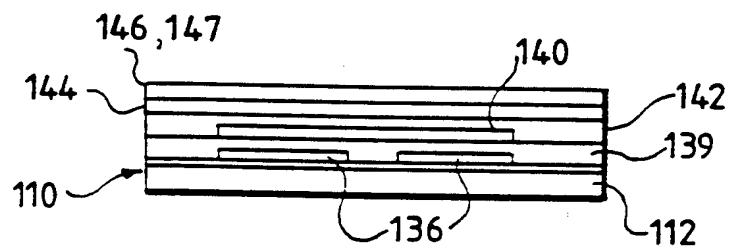


Fig.19.

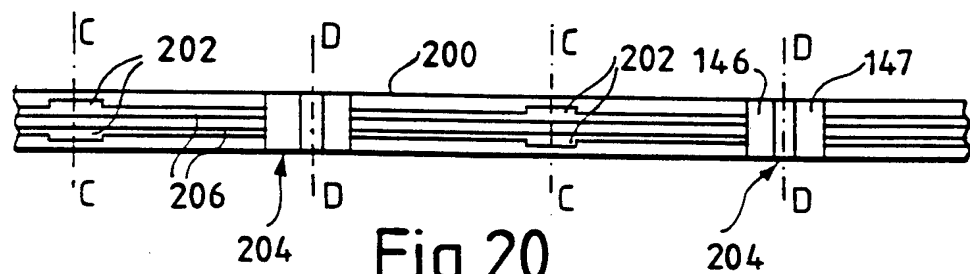


Fig.20.

INTERNATIONAL SEARCH REPORT

In onal Application No

PCT/GB 94/01690

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 G01N27/28 G01N27/416

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ANALYTICAL CHEMISTRY, vol.59, 1987, COLUMBUS US pages 2692 - 2699 S.A.SCHUETTE ; R.L.MCCREARY 'hydrodynamically modulated alternating current voltammetry' cited in the application ---	1,6,7, 10,14
Y	WO,A,92 21961 (CAPTEUR SENSORS & ANALYSERS LTD) 10 December 1992 cited in the application see the whole document ---	1,6,7, 10,14
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

6 December 1994

Date of mailing of the international search report

13.12.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+31-70) 340-3016

Authorized officer

BROCK, T

INTERNATIONAL SEARCH REPORT

Int. Appl. No.
PCT/GB 94/01690

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>REVIEW OF SCIENTIFIC INSTRUMENTS, MAY 1988, USA, VOL. 59, NR. 5, PAGE(S) 787 - 792, ISSN 0034-6748</p> <p>SCHEFFEY C 'Two approaches to construction of vibrating probes for electrical current measurement in solution'</p> <p>---</p>	1,10,14
A	<p>IEEE TRANSACTIONS ON BIO-MEDICAL ENGINEERING, vol.BME-34, no.9, September 1987, NEW YORK US</p> <p>pages 657 - 663</p> <p>B.C.TOWE 'vibration of polarographic oxygen electrodes by piezoelectric elements : a method of reducing flow artifacts'</p> <p>---</p>	1,10,14
A	<p>EP,A,0 504 730 (SEIKO INSTRUMENTS INC) 23 September 1992</p> <p>see the whole document</p> <p>---</p>	1,10,14
A	<p>DE,A,40 40 293 (PÄDAGOGISCHE HOCHSCHULE HALLE-KÖTHEN) 25 June 1992</p> <p>see the whole document</p> <p>---</p>	1,10,14
A	<p>EP,A,0 122 420 (BOEHRINGER MANNHEIM GMBH) 24 October 1984</p> <p>see the whole document</p> <p>-----</p>	1,10,14

INTERNATIONAL SEARCH REPORT

information on patent family members

In. onal Application No

PCT/GB 94/01690

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9221961	10-12-92	EP-A- 0587665 JP-T- 6508432	23-03-94 22-09-94
EP-A-0504730	23-09-92	JP-A- 4294245 JP-A- 4310842 US-A- 5334303	19-10-92 02-11-92 02-08-94
DE-A-4040293	25-06-92	NONE	
EP-A-0122420	24-10-84	DE-A- 3312923 AU-A- 2582184 DE-A- 3485840 JP-C- 1774468 JP-B- 4065975 JP-A- 59190653	11-10-84 18-10-84 03-09-92 14-07-93 21-10-92 29-10-84